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Investigation of Scrapie-Infected Hamster Nervous Tissue by Infrared Microscopy

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Beamline(s): U10B

Scrapie is a fatal neurodegenerative disease in sheep and goats which belongs to the family of prion diseases. These diseases, among them also bovine spongiform encephalopathy and human Creutzfeldt-Jakob disease, possess a common, very specific feature: deposits of the pathological prion protein (PrP^{Sc}), which accumulates in many regions of the central and the peripheral nervous system [1]. Since PrP^{Sc} aggregates show a high content of β-sheet structures, they should be detectable in sections of nervous tissue by changes in the amide bands of IR spectra [2]. However, the accumulations of PrP^{Sc} are very small, so that all IR microspectroscopic approaches using a standard thermal light source have failed to detect PrP^{Sc} because of the poor spatial resolution of these systems. We therefore used the synchrotron light of NSLS beamline U10B to investigate sections of nervous tissue from hamsters that were infected with an experimental strain (263 K) of scrapie, and from healthy hamsters. In our experiment, dorsal root ganglia were cryo-sectioned. Two adjacent sections were stained with the prion-specific antibody mAb 3F4 and mounted to BaF_2 windows for IR microscopy, respectively. Single neurons showing PrP^{Sc}-positive staining in the adjacent section as well as single neurons of uninfected ganglia were investigated by IR spectral mapping.

Fig. 1A contains photomicrographs of two neurons from an uninfected ganglion and an IR spectrum obtained from these cells (Fig. 1B). Functional group maps were constructed with different parameters determined from the spectra (see Fig. 1C). Results show that IR images of single neurons can be assembled based on the spatially resolved information contained in the spectra. Furthermore, they indicate that the spectral features of protein absorption differ between different locations in the infected neurons. The results suggest that scrapie-induced changes of the protein distribution and composition can successfully be investigated using IR spectroscopy at high spatial resolution and will help to elucidate more biochemical aspects of prion diseases.

References

1. McBride, P.A. and M. Beekes, *Pathological PrP is abundant in sympathetic and sensory ganglia of hamsters fed with scrapie.* Neuroscience Letters, 1999. **265**: p. 135-138.
2. Pan, K.M., et al., *Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins.* Proc. Natl. Acad. Sci. USA, 1993. **90**: p. 10962-10966.

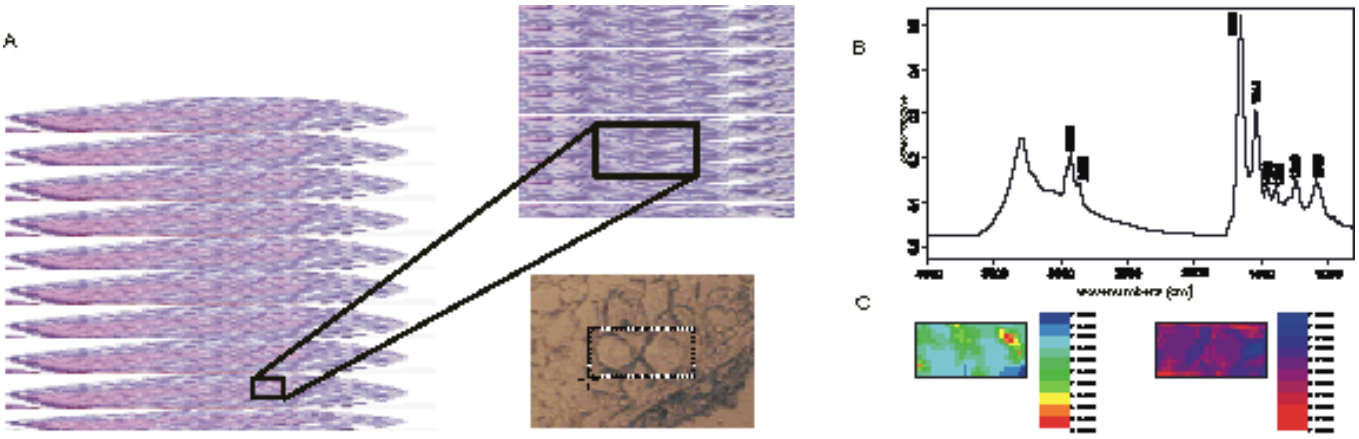

Figure 1: A) Light microscopic images of the section of an uninfected ganglion, stained with hematoxylin and eosin at two magnifications and adjacent, unstained section as seen through the microscope at beamline U10B. The area investigated by IR mapping is indicated. B) Spectrum from an area of 10 x 10 microns of a healthy ganglion cell. C) Chemical maps of the mapped area reconstructed with two parameters. Left: Protein/ lipid ratio, right: integral intensity of the amide II band (1544 cm^{-1}).